REMARKS

Claims 1-22 and 30 are pending. With entry of the present Amendment, Claims 1, 7, 11 and 15 are amended and Claims 4-6 and 30 are cancelled.

Claims 1, 7, 11 and 15 have been amended to remove the objected phrase "improved thermostable cellulase activity". Claims 1 and 15 have been further amended to include the functional language of Claim 30. Specifically, the claims have been amended to recite the thermostable cellulase is more soluble and has a specific acitivity that is at least two times greater than the specific activity of the polypeptide having the full-length sequence of SEQ ID NO: 2 using carboxymethyl cellulose as substrate. Support for this amendment is found in the Specification at page 3, lines 6-9 and page 5, lines 23 to 24. The Claims are also amended to refer to specific sequences of SEQ ID NO: 2 and SEQ ID NO: 3. Support for these amendments is found on page 2, line 19 to page 3, line 11. No new matter has been added.

Applicants have made enzymes with improved activity by deleting certain amino acid residues from the amino acid sequence set forth in SEQ ID NO: 2. The specific embodiments described in the Examples disclose enzyme variants where either 17 or 38 amino acid residues have been deleted from the N-terminus of SEQ ID NO: 2,. These are referred to as Cel12A(sp-) and δCel12A, respectively This is contrary to the Examiner's statement that Applicants have made an enzyme with 40 amino acid residues have been deleted. Applicants variants show enhanced activity, improved stability and much more solubility than the full length enzyme. However, Applicants note an error is in Table 1 on p. 30 in the Application. The second enzyme should be designated δCel12AH (the –38 a.a. variant), not Cel12AH. Applicants have corrected this typographical error in the Specification. Support for the amendments can be found in the Specification on page 28, line 4 to page 29, line 4. Support is also shown in Table 2 (line 2) in Exhibit 2, filed with the Amendment dated February 24, 2003.

Rejection of Claims 1-22 and Claim 30 under 35 U.S.C. §112, Second Paragraph

Claims 1-22 stand rejected under 35 U.S.C. § 112, second paragrah, as being indefinite for failing to particulary point out and distinctly claim the subject matter which Applicants regard

as the invention. In particular, the Examiner has objected to the phrase "improved thermostable cellulase activity".

Claims 1, 7, 11 and 15 have been amended to remove the objected phrase. As amended, the Claims now specify that the polypeptides encoded by the claimed nucleic acids are "more soluble and have specific cellulase activity that is at least two times greater than the specific activity of the polypeptide having the sequence of SEQ ID NO: 2 using carboxymethyl cellulose as its substrate". Claims 1 and 15 have been further amended to add language describing the variant enzymatic activity and solubility. Claims 7 and 11 refer to specific nucleic acid sequences demonstrated as having these desired properties. Thus, the claims are definite as amended.

Further, Claims 1-22 and Claim 30 have been rejected as indefinite for the phrase "corresponding to the position one to about 40 in the corresponding SEQ ID NO: 2" and the phrase "about 85% identity compared to SEQ ID NO: 2".

Applicants have removed these phrases from the claims and included reference to SEQ ID NO: 2, thus obviating the rejection.

In view of the amendments to the claims, the claims are definite and clearly define the subject matter which Applicants' regard as the invention. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 1-13 and 15-20 U.S.C. §112, First Paragraph

Claims 1-13 and 15-20 are rejected under 35 U.S.C. §112, first paragraph, because the Examiner states that the specification does not reasonably provide enablement for any variant DNA sequence from any source encoding a variant glycosyl hydrolase of family 12, and does not enable a person skilled in the art to which it pertains to use the invention commensurate in scope with these claims. Applicants respectfully disagree.

The Claims have been amended herein to recite structural and functional attributes that characterize the genus of variants claimed. For example, in the amended claims, the variants have been structurally described by particular sequences recited; *i.e.,* SEQ ID NO: 2 and SEQ ID NO: 3. In some embodiments, the variants are further described as more stable and having at

least twice the specific activity as the polypeptide encoded by SEQ ID NO: 2 using carboxymethyl cellulose as substrate. Applicants have removed the objected language of the variants being 85% identical to SEQ ID NO: 2. As amended, the variants comprise SEQ ID NO 2 or are encoded by specific sequences of SEQ ID NO: 3.

The Specification discloses methods suitable for preparing truncated nucleic acids encoding thermostable cellulases belonging to the glycosyl hydrolase family 12 and polypeptides encoding such thermostable cellulases and for determining the enzymatic activity of the resultant polypeptides. Using the teachings and sequences described in the Specification and specifically provided in the amended claims as a guide, one skilled in the art would certainly be capable of making additions, deletions and substitutions within the provided sequence framework described in the amended claims. Additionally, a method to test the resultant variants in an enzymatic assay is detailed in the Exemplification. Therefore, the Specification, in view of the amended claims, details the necessary knowledge to determine a variant's inclusion in the genus. As previously argued, such experimentation is routinely undertaken in the art and would not be considered undue. In view of these points, the claims as amended are not overly broad and considerable direction is provided.

Contrary to the Examiner's assertion, Applicants' disclosure is <u>not</u> limited to a single nucleotide encoding the polypeptide in which all of the first 40 N-terminal amino acids are deleted. Applicants demonstrated different variants (for example, one with 17 amino acids deleted and the other with 38 amino acids deleted) having improved properties over the wild type enzyme. These enzymes have greater solubility and higher specific activity compared to the wild-type enzyme.

Applicants are not required to demostrate by example all embodiments of their invention but to provide direction to one of ordinary skill in the art to make and use the invention without undue experimentation. Applicants have met this criteria. Determining other enzymes encompassed by Applicants' amended claims is straightforward using the teachings and examples provided in the Specification. No undue burden is placed upon one of ordinary skill in the art to make and use Applicants' invention. Thus, Applicants submit that the claimed invention as amended is enabled, and respectfully request reconsideration and withdrawal of the rejection.

Rejection of Claims 1-22 and 30 under 35 U.S.C. §103(a)

Claims 1-22 and 30 stand rejected under 35 U.S.C. § 103 (a) as obvious over Halldorsdottir *et al.* and the enclosed sequence and Ohmiya *et al.* (*J. Bacteriol.*, 173(2):636-641(1991)).

The Examiner submits that Halldorsdottir *et al.* teach the cloning, sequencing and overexpression of a *R. marinus* gene encoding a thermostable cellulase, and that the reference provides the polynucleotide sequence as a GenBank deposit. The Examiner further states that N-terminal truncation of the signal sequence in a bacterial endoglucanase resulted in higher cellulase activity compared to the wild-type enzyme. Additionally, the Examiner states that by combining the teachings of the two references, the invention would have been *prima facie* obvious to one of ordinary skill in the art. Applicants respectfully disagree with the Examiner's conclusions.

Applicants' invention pertains to the unexpected discovery that removal of the N-terminal hydrophobic region and/or the linker moiety of glycosyl hydrolases of family 12, as described by SEQ ID NO: 2, yielded polypeptides of thermostable cellulases with superior catalytic and physical properties when compared to the native full-length polypeptide. These variant polypeptides can now be produced efficiently in recombinant organisms with superior properties, such as increased stability, solubility and specific activity. See Specification, page 5, lines 13-20. The variant polypeptides have pH optima and substrate specificities similar to full-length polypeptides but their specific activities were improved - surprisingly three-fold higher than the specific activity of the full length enzyme. The variant polypeptides were also shown to be more stable than the full length enzyme. These unexpected superior characteristics allow for increased versatility in commercial applications. For example, the improvement in specific activity allows one to reduce the amounts of enzyme needed in reactions, thereby providing cost advantages.

Halldorsdottir *et al.* teach cloning, sequencing and overexpression of a *Rhodothermus marinus* gene encoding a thermostable cellulase of glycosyl hydrolase family 12. Furthermore, vectors, host cells and methods of producing the cellulase in the host cells are provided. However, there is no teaching or suggestion in Halldordottir *et al.* of the benefit of N-terminal

truncations of the cellulases. Therefore, this reference only teaches characterization and methods of producing the wild-type form of the cellulase from *R. marinus*.

Applicants did not merely retain the catalytic activity with the variants produced from less than the full length gene but showed increased catalytic activity. Applicants demonstrated that the N-terminal end was detrimental to the cells and further that removal of amino acids at the N-terminal end produced variants with superior properties, including over a three-fold increase in catalytic activity.

In the Exemplification of the instant application, the full length enzyme was shown to be inefficiently expressed. The *E. coli* cells lysed soon after induction of the full length enzyme indicating that the full length enzyme was cytotoxic. Further, the full length enzyme had reduced stability losing activity when stored over a period of time. Additionally, the full length enzyme was shown to aggregate and displayed poor thermostablity when the enzyme was heat treated at 65°C, which is the physiological temperature of the host organism *R. marinus*. See Specification, page 5, lines 2-4. Applicants discovered a way to increase expression, decrease aggregation, increase enzymatic activity and increase stability of the polypeptides by removal of amino acids from the N-terminal end of the polypeptide. The polypeptides of the invention possess improved characteristics, such as increased stability (*e.g.*, thermal stability, detergent stability), increased solubility in aqueous solvents, increased catalytic activity (*e.g.*, specific activity, catalytic rate) and/or reduced cytotoxicity relative to the native or full-length thermostable cellulase, but retain the substrate specificity of the native or full-length cellulase. See Specification page 8, lines 19-26. These beneficial properties and increased yield of the thermostable cellulase are not taught or suggested in Halldorsdottir *et al.* There is no suggestion in the Halldorsdottir *et al.* reference that a variant of less than the complete gene would yield an enzyme with any of the superior properties discussed above.

Ohmiya *et al.* discuss and disclose variants of an endoglucanase by gene truncation. The enzymes are derived from a mesophilic bacterium, *Ruminococcus albus*. The properties and characteristics of a mesophilic enzyme are in contrast to Applicants' enzymes that are thermostable. The mature enzyme (EgI) described by Ohmiya *et al.* has an optimum temperature of 37°C. The variant enzymes described in Ohmiya *et al.* vary widely in activity and stability from each other. In certain instances, the N-terminal truncations yielded an enlzyme with

minimal activity and in other instances the truncation yielded an enzyme with higher activity when compared to the wild-type. But Ohmiya *et al.* report the active truncated variants (EgI-1 and EgI-2) have a *lower* optimum temperatures compared to the wild-type enzyme. (See page 640, 1st. Column). The optimal temperature decreased from 37°C to 30°C . Ohmiya further states:

> "the activity [of EgI-1 and EgI-2] decreased remarkably at a temperature higher than 40°C. Although EgI retained 10% of the initial activity even at 100°C, N-terminal truncated endoglucanases lost all activity at approximately 55°C (Fig. 6C). It seems that the N-terminal amino acid sequence of EgI makes an important contribution to the stability of endoglucanase" (p. 640, bridging paragraph of Col. 1 and Col. 2, emphasis added).

For modifying thermophilic enzymes to obtain thermostable cellulases, one of skill in the art would not look to the teachings of a mesophilic organism that taught deletion of the N-terminus is deliterious to temperature optimum. The present invention is directed to thermostable cellulases. Thermostability, *e.g.,* temperature stability, is one of the features of Applicants' claimed invention.

Applicants describe N-truncated thermostable variants of the *R. marinus* endoglucanase with enhanced activity. Importantly, these truncated variants also have much higher solubility and stability than the wild-type enzyme. Extensive aggregation of the wild-type full-length enzyme was observed at elevated temperature (above about 65°-70°C) (see p. 31 of the Specification) and the full-length enzyme is poorly expressed and causes cell lysis. The truncated variants overcome the aggregation problem. Additionally, the truncated variants as described by Applicants have improved stability. Surprisingly, some retain their activity after 16 hours at 85°C.

Based on the arguments above, Applicants respectfully disagree that the combination of the teachings of Halldorsdottir *et al.* and Ohmiya *et al.*, it would have been obvious to modify the Cel12 sequence from Halldorsdottir *et al.* to obtain the improved enzymes of the present invention. Ohmiya *et al.* specifically state that their

truncated endoglucanases lost all activity at moderately elevated temperatures (above 55°C) whereas the non-truncated enzyme retains 10% activity at 100°C. Ohmiya *et al.* also state that the N-terminal region is important for the stability of the enzyme. Thus, although Ohmiya *et al.* disclose that N-truncations of the mesophilic EgI endogluganase can enhance activity they also indicate that such truncations may *reduce* stability, in particular temperature stability and optimum. Reductions in stability are detrimental to thermostable enzymes that are valuable because they are stable, *e.g.,* they operate at high temperatures. Thus, Ohmiya *et al.* teach away from Applicants' invention.

Further, Ohmiya *et al.* do not discuss potential effects of N-terminal truncations on solubility and, in fact, solubility of the full-length EgI does not seem to be a problem. This is in contrast to the polypeptide of the present invention where much improved solubility is an important advantage of the N-truncated variants and reflected in the amended claims.

In determining patentability under 35 U.S.C. § 103, a prior art reference must be considered as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.* 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), cert. denied, 469 U.S. 851 (1984). The full disclosure of Ohmiya *et al.* as described above, therefore, teaches away from Applicants' invention.

The Examiner has failed to establish a *prima facie* case of obviousness. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must be found in the prior art and not based on applicant's disclosure. *In re Vaeck,* 947 F. 2d 488, 20 U.S.P.Q. 2d 1438 (Fed. Cir. 1991).

Applicants amended claims are directed to thermostable enzymes that are more soluble and have specific cellulase activity that is at least two times greater than the wild-type enzyme. As the prior art fails to teach or suggest these features of the claimed invention

and actually teaches away from what is claimed, the § 103 rejections are overcome and the present invention should be allowed. Reconsideration and withdrawal of the rejections are respectfully requested.

CONCLUSION

In summary, it is concluded that the art cited by the Examiner does not render obvious Applicants' claimed invention. Accordingly, reconsideration and withdrawal of the rejections are respectfully requested. Applicants' Agent respectfully requests a telephone conference with the Examiner before the issuance of a subsequent Office Action to further expedite the prosecution of this Application. Please call the undersigned or Alice O. Carroll, Esq. at the number given below.

Respectfully submitted,

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